## AMENDMENTS TO THE SPECIFICATION

*In the Sequence Listing:*

A Substitute Sequence Listing is submitted herewith. Please enter the Substitute Sequence Listing into the record. Please delete the Sequence Listing filed in the above-captioned patent application on October 3, 2001.

*In the Drawings:*

Corrected drawings for Figures 1-10 as required by the Examiner are submitted herewith. Please enter corrected drawings into the record.

*In the Specification:*

Please amend the specification as originally filed at pages 5 to 6, pages 9 to 10, and pages 12 to 14 by entering the replacement sections presented below.

*At pages 5 to 6 of the specification please replace the entire Summary of the Invention section starting on page 5 at line 4 and ending on page 6 at line 25 with the following replacement section:*

### SUMMARY OF THE INVENTION

According to the present invention there is provided, peptides which are substantially homologous in sequence to a part of the amino acid sequence of a human myelin basic protein. These peptides are capable of neutralizing or modulating the production of anti-MBP.

According to the present invention the peptides are of the formula:

$R_1$-Val-His-Phe-Phe-Lys-Asn-Ile-$R_2$

and salts thereof, wherein Val-His-Phe-Phe-Lys-Asn-Ile- is amino acid residues 87-93 of SEQ ID NO:1, and wherein $R_1$ and $R_2$ are independently selected from the group consisting of hydrogen, hydroxy, the residue of an amino acid and the residue of a polypeptide; provided that $R_1$ and $R_2$ are not both hydrogen or hydroxyl at the same time. The peptide can contain substitutions, deletions or additions thereof, provided that the peptide maintains its function of neutralizing or modulating the production of anti-MBP.

Examples of said peptides are selected from:

**MBP75-95** (amino acid residues 75-95 of SEQ ID NO:1)

Lys Ser His Gly Arg Thr Gln Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val Thr

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**MBP64-78** (amino acid residues 64-78 of SEQ ID NO:1)

Ala Arg Thr Ala His Tyr Gly Ser Leu Pro Gln Lys Ser His Gly

**MBP61-75** (amino acid residues 61-75 of SEQ ID NO:1)

His His Pro Ala Arg Thr Ala His Tyr Gly Ser Leu Pro Gln Lys

**MBP69-83** (amino acid residues 69-83 of SEQ ID NO:1)

Tyr Gly Ser Leu Pro Gln Lys Ser His Gly Arg Thr Gln Asp Glu

**MBP80-97** (amino acid residues 80-97 of SEQ ID NO:1)

Thr Gln Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val Thr Pro Arg

**MBP91-106** (amino acid residues 91-106 of SEQ ID NO:1)

Lys Asn Ile Val Thr Pro Arg Thr Pro Pro Pro Ser Gln Gly Lys Gly

**MBP84-93** (amino acid residues 84-93 of SEQ ID NO:1)

Asn-Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile

**MBP85-94** (amino acid residues 85-94 of SEQ ID NO:1)

Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val

**MBP86-95** (amino acid residues 86-95 of SEQ ID NO:1)

Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr

**MBP87-96** (amino acid residues 87-96 of SEQ ID NO:1)

Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro

Further according to the present invention there is provided pharmaceutical compositions, which comprises as an active ingredient a peptide as described above, either alone or in combination, in admixture with a pharmaceutical acceptable carrier.

B 1

Further according to the present invention, there is provided a method of treating multiple sclerosis comprising administering an effective amount of a peptide as, described above, either alone or in combination to effectively neutralize or modulate the production of anti-human myelin basic protein.

*At pages 9 to 10 of the specification, replace the paragraphs of the Detailed Description of the Invention starting with the paragraph beginning on page 9 at line 29 and ending on page 10 at line 27 with the following replacement paragraphs:*

Based on the present invention, on the basis of the competitive inhibition assays using a series of 41 decapeptides, the MBP epitope for MS anti-MBP has been localized to an area between amino acid 82 and amino acid 98, greater than 40% inhibition of bound anti-MBP and greater than 60% inhibition of free anti-MBP. Based on the highest level of inhibition, the MBP epitope for MS anti-MBP is probably between amino acid 84 and amino acid 96. The smallest common region of the effective decapeptides is from amino acid 87 to amino acid 93. Thus, according to the present invention, the peptides can be illustrated by the following formula:

B 2

$R_1$ -Val-His-Phe-Phe-Lys-Asn-Ile- $R_2$

and salts thereof, wherein Val-His-Phe-Phe-Lys-Asn-Ile- is amino acid residues 87-93 of SEQ ID NO:1, and wherein $R_1$ and $R_2$ are independently selected from the group consisting of hydrogen, hydroxy, the residue of an amino acid and the residue of a polypeptide; provided that $R_1$ and $R_2$ are not both hydrogen or hydroxyl at the same time.

The 7 amino acids spanning amino acid position 87 to 93 would probably not be large enough to effectively bind anti-MBP. Thus, $R_1$ and $R_2$ cannot both be hydrogen or both be hydroxy at the same time.

B 2

When $R_1$ and $R_2$ is an amino acid, the amino acid can be selected from naturally occurring amino acids. $R_1$ and $R_2$ are not restricted to the amino acids occurring upstream or downstream of Val87 and Ile93 in the human myelin basic protein, as shown in SEQID NO: 1. Various modification, including substitutions, additions or deletions in the upstream and downstream sequences of $R_1$ and $R_2$ can be used. In addition, modification, including substitutions, additions or deletions can be made to the sequence -Val-His-Phe-Phe-Lys-Asn-Ile (amino acid residues 87-93 of SEQ ID NO:1), provided that the peptides so produced still function in their intended use; i.e., to neutralize or modulate the production of antibodies to myelin basic protein.

*At pages 12 to 14 of the specification, replace the paragraphs of the Detailed Description of the Invention starting on page 12 at line 7 and ending on page 14 at line 4, with the following replacement paragraphs:*

B 3

According to one embodiment of the present invention it has been determined that selected peptides substantially corresponding to the amino acid sequence of the h-MBP are effective in neutralizing or modulating the production of anti-MBP. These peptides correspond to the amino acid sequence of the h-MBP from about amino acid residue 61 to about amino acid residue 106. In one example these peptides correspond to the amino acid sequence of the h-MBP from about amino acid residue 75 to about amino acid residue 106, when the peptides are used for the neutralization of free anti-MBP. In a further example, these peptides correspond to the amino acid sequence of the h-MBP from about amino acid residue 82 to about amino acid residue 99, when the peptides are used for the neutralization or modulation of the production of bound anti-

MBP. Therefore the peptides are selected from 10 amino acid residues to 25 amino acid residues taken from a continuous amino acid sequence within the sequence shown below (amino acid residues 61-106 of SEQ ID NO:1), provided that said sequence can neutralize or modulate the production of the anti-myelin basic protein.

**Amino acid residues 61-106 of SEQ ID NO:1**

61

His His Pro Ala Arg Thr Ala His Tyr Gly Ser Leu Pro Gln Lys Ser His Gly
Arg Thr Gln Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val Thr Pro
Arg Thr Pro Pro Pro Ser Gln Gly Lys Gly

106

Examples of peptides are selected from the group consisting of:

**MBP61-75** (amino acid residues 61-75 of SEQ ID NO:1)
His His Pro Ala Arg Thr Ala His Tyr Gly Ser Leu Pro Gln Lys
**MBP64-78** (amino acid residues 64-78 of SEQ ID NO:1)
Ala Arg Thr Ala His Tyr Gly Ser Leu Pro Gln Lys Ser His Gly
**MBP69-83** (amino acid residues 69-83 of SEQ ID NO:1)
Tyr Gly Ser Leu Pro Gln Lys Ser His Gly Arg Thr Gln Asp Glu
**MBP75-95** (amino acid residues 75-95 of SEQ ID NO:1)
Lys Ser His Gly Arg Thr Gln Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val Thr
**MBP80-97** (amino acid residues 80-97 of SEQ ID NO:1)
Thr Gln Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val Thr Pro Arg

In one embodiment of the present invention, the peptides are represented by the formula:

$$R_1\text{-Val-His-Phe-Phe-Lys-Asn-Ile-}R_2$$

and salts thereof, wherein Val-His-Phe-Phe-Lys-Asn-Ile is amino acid residues 87-93 of SEQ ID NO:1, and wherein $R_1$ and $R_2$ are independently selected from the group

consisting of hydrogen, hydroxy, the residue of an amino acid and the residue of a polypeptide; provided that $R_1$ and $R_2$ are not both hydrogen or hydroxyl at the same time. The peptide can contain substitutions, deletions or additions thereof, provided that the peptide maintains its function of neutralizing or modulating the production of anti-MBP.

Examples of peptides are selected from:

B 3

**MBP84-93** (amino acid residues 84-93 of SEQ ID NO:1)

Asn-Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile

**MBP85-94** (amino acid residues 85-94 of SEQ ID NO:1)

Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val

**MBP85-94** (amino acid residues 85-94 of SEQ ID NO:1)

Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr

**MBP87-96** (amino acid residues 87-96 of SEQ ID NO:1)

Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro

**MBP91-106** (amino acid residues 91-106 of SEQ ID NO:1)

Lys Asn Ile Val Thr Pro Arg Thr Pro Pro Pro Ser Gln Gly Lys Gly

#10



Further the peptides of the present invention were investigated to determine their effectiveness in binding or modulating the production of MS anti-MBP *in vivo*.

## SUMMARY OF INVENTION

According to the present invention there is provided, peptides which are substantially homologous in sequence to a part of the amino acid sequence of a human myelin basic protein. These peptides are capable of neutralizing or modulating the production of anti-MBP.

According to the present invention the peptides are of the formula:

$R_1$-Val-His-Phe-Phe-Lys-Asn-Ile-$R_2$

(wherein Val-His-Phe-Phe-Lys-Asn-Ile is amino acid residues 87-93 of SEQ ID NO:1, and)

and salts thereof, wherein $R_1$ and $R_2$ are independently selected from the group consisting of hydrogen, hydroxy, the residue of an amino acid and the residue of a polypeptide; provided that $R_1$ and $R_2$ are not both hydrogen or hydroxyl at the same time. The peptide can contain substitutions, deletions or additions thereof, provided that the peptide maintains its function of neutralizing or modulating the production of anti-MBP.

Examples of said peptides are selected from:

**MBP75-95** (amino acid residues 75-95 of SEQ ID NO: 1)
Lys Ser His Gly Arg Thr Gln Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val Thr

**MBP64-78** (amino acid residues 64-78 of SEQ ID NO:1)
Ala Arg Thr Ala His Tyr Gly Ser Leu Pro Gln Lys Ser His Gly

**MBP61-75** (amino acid residues 61-75 of SEQ ID NO:1)
His His Pro Ala Arg Thr Ala His Tyr Gly Ser Leu Pro Gln Lys

**MBP69-83** (amino acid residues 69-83 of SEQ ID NO:1)
Tyr Gly Ser Leu Pro Gln Lys Ser His Gly Arg Thr Gln Asp Glu
**MBP80-97** (amino acid residues 80-97 of SEQ ID NO:1)
Thr Gln Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val Thr Pro Arg
**MBP91-106** (amino acid residues 91-106 of SEQ ID NO:1)
Lys Asn Ile Val Thr Pro Arg Thr Pro Pro Pro Ser Gln Gly Lys Gly
**MBP84-93** (amino acid residues 84-93 of SEQ ID NO:1)
Asn-Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile
**MBP85-94** (amino acid residues 85-94 of SEQ ID NO:1)
Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val
**MBP86-95** (amino acid residues 86-95 of SEQ ID NO:1)
Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr
**MBP87-96** (amino acid residues 87-96 of SEQ ID NO:1)
Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro

Further according to the present invention there is provided pharmaceutical compositions, which comprises as an active ingredient a peptide as described above, either alone or in combination, in admixture with a pharmaceutical acceptable carrier.

Further according to the present invention, there is provided a method of treating multiple sclerosis comprising administering an effective amount of a peptide as, described above, either alone or in combination to effectively neutralize or modulate the production of anti-human myelin basic protein.

## BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the localization of eighteen synthetic peptides (small numbers) in relation to the intact human-MBP molecule. Peptides are represented by vertical bars placed next to their corresponding region on the MBP molecule. Large numbers represent amino acid residues on human MBP.

60% inhibition of free anti-MBP. Based on the highest level of inhibition, the MBP epitope for MS anti-MBP is probably between amino acid 84 and amino acid 96. The smallest common region of the effective decapeptides is from amino acid 87 to amino acid 93. Thus, according to the present invention, the peptides can be illustrated by the following formula:

$R_1$-Val-His-Phe-Phe-Lys-Asn-Ile-$R_2$

wherein Val-His-Phe-Phe-Lys-Asn-Ile is amino acid residues 87-93 of SEQ ID NO:1, and

and salts thereof, wherein $R_1$ and $R_2$ are independently selected from the group consisting of hydrogen, hydroxy, the residue of an amino acid and the residue of a polypeptide; provided that $R_1$ and $R_2$ are not both hydrogen or hydroxyl at the same time.

The 7 amino acids spanning amino acid position 87 to 93 would probably not be large enough to effectively bind anti-MBP. Thus, $R_1$ and $R_2$ cannot both be hydrogen or both be hydroxy at the same time.

When $R_1$ or $R_2$ is an amino acid, the amino acid can be selected from naturally occurring amino acids. $R_1$ or $R_2$ are not restricted to the amino acids occurring upstream or downstream of Val87 and Ile93 in the human myelin basic protein, as shown in SEQID NO: 1. Various modification, including substitutions, additions or deletions in the upstream and downstream sequences of $R_1$ and $R_2$ can be used. In addition, modification, including substitutions, additions or deletions can be made to the sequence -Val-His-Phe-Phe-Lys-Asn-Ile, (amino acid residues 87-93 of SEQ ID NO:1) provided that the peptides so produced still function in their intended use; i.e., to neutralize or modulate the production of antibodies to myelin basic protein.

The term "residue of polypeptide" or "polypeptide residue" is meant to include di, tri, and higher polypeptides including proteins or fragments thereof. As above, when $R_1$ or $R_2$ is a polypeptide residue, $R_1$ or $R_2$ are not limited to the peptides occurring upstream or downstream of

peptides can be at least 10 amino acids in length. In one example of the present invention the peptides can be from about 10 amino acid residues to about 25 amino acid residues. If the peptides of the present invention are used as part of a fusion protein, the overall size of the peptide can be much larger.

According to one embodiment of the present invention it has been determined that selected peptides substantially corresponding to the amino acid sequence of the h-MBP are effective in neutralizing or modulating the production of anti-MBP. These peptides correspond to the amino acid sequence of the h-MBP from about amino acid residue 61 to about amino acid residue 106. In one example these peptides correspond to the amino acid sequence of the h-MBP from about amino acid residue 75 to about amino acid residue 106, when the peptides are used for the neutralization of free anti-MBP. In a further example, these peptides correspond to the amino acid sequence of the h-MBP from about amino acid residue 82 to about amino acid residue 99, when the peptides are used for the neutralization or modulation of the production of bound anti-MBP. Therefore the peptides are selected from 10 amino acid residues to 25 amino acid residues taken from a continuous amino acid sequence within the sequence shown below (SEQID NO:1), provided that said sequence can neutralize or modulate the production of the anti-myelin basic protein.

Amino acid residues 61-106 of SEQID NO:1

Amino acid residues 61-106 of SEQID NO: 1

61

His His Pro Ala Arg Thr Ala His Tyr Gly Ser Leu Pro Gln Lys Ser His Gly Arg Thr Gln Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro Pro Ser Gln Gly Lys Gly

106

Examples of peptides are selected from the group consisting of:

**MBP61-75** (amino acid residues 61-75 of SEQ ID NO: 1)

His His Pro Ala Arg Thr Ala His Tyr Gly Ser Leu Pro Gln Lys

**MBP64-78** (amino acid residues 64-78 of SEQ ID NO: 1)

Ala Arg Thr Ala His Tyr Gly Ser Leu Pro Gln Lys Ser His Gly

**MBP69-83** (amino acid residues 69-83 of SEQ ID NO: 1)

Tyr Gly Ser Leu Pro Gln Lys Ser His Gly Arg Thr Gln Asp Glu

**MBP75-95** (amino acid residues 75-95 of SEQ ID NO: 1)

Lys Ser His Gly Arg Thr Gln Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val Thr

**MBP80-97** (amino acid residues 80-97 of SEQ ID NO: 1)

Thr Gln Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val Thr Pro Arg

**MBP91-106** (amino acid residues 91-106 of SEQ ID NO: 1)

Lys Asn Ile Val Thr Pro Arg Thr Pro Pro Pro Ser Gln Gly Lys Gly

In one embodiment of the present invention, the peptides are represented by the formula:

$$R_1\text{-Val-His-Phe-Phe-Lys-Asn-Ile-}R_2$$

and salts thereof, wherein $R_1$ and $R_2$ are independently selected from the group consisting of hydrogen, hydroxy, the residue of an amino acid and the residue of a polypeptide; provided that $R_1$ and $R_2$ are not both hydrogen or hydroxyl at the same time. The peptide can contain substitutions, deletions or additions thereof, provided that the peptide maintains its function of neutralizing or modulating the production of anti-MBP.

Examples of peptides are selected from:

**MBP84-93** (amino acid residues 84-93 of SEQ ID NO: 1)

Asn-Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile

**MBP85-94** (amino acid residues 85-94 of SEQ ID NO: 1)

Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val

**MBP86-95** (amino acid residues 86-95 of SEQ ID NO:1)

Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr

**MBP87-96** (amino acid residues 87-96 of SEQ ID NO:1)

Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro

The potential role of anti-MBP in the pathogenesis of MS continues to be explored. Increased anti-MBP titers in patients with active MS were initially reported by Panitch et al (Panitch, H.S., Hooper, C.S., and Johnson, K.P., Arch Neurol 37:206-209, 1980) who used a solid phase radioimmunoassay with guinea-pig MBP. Patients with acute MS relapses have usually increased anti-MBP predominantly in free form, while some patients in clinical remission may have undetectable anti-MBP levels. During the transition phase from an acute relapse to remission, titers of free anti-MBP progressively decrease over weeks or months, while bound fractions of the antibody rise as compared to their initial value. In other patients in remission, it is possible to observe low titers of free and bound anti-MBP, usually with a F/B ratio below unity, suggesting that anti-MBP neutralizing antibody(ies) are bound to anti-MBP. Occasionally, patients who fit the criteria of clinically definite MS or patients who had neuropathologically confirmed MS had undetectable anti-MBP during active phases of their disease. It is possible that such patients have antibodies to other myelin proteins. The absence of a specific antibody scenario does not negate the potential importance of anti-MBP in the mechanism of demyelination in the majority of MS patients.

Recently, an MBP antibody cascade was observed in the IgG fraction purified from MS CSF (Warren, K.G. and Catz, I., J Neurol Sci 96:19-27, 1990). Primary antibodies to MBP in both free and bound forms occur in association with active disease: F/B ratios are above unity in patients with acute relapses, and below unity in patients with chronic progressive disease (Warren, K.G. and Catz, I., Ann Neurol 209:20-25, 1986; Catz, I. and Warren, K.G., Can J Neurol Sci 13:21-24, 1986; and Warren, K.G. and Catz,